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Improved recovery of enzyme activity after elcctrophoresis

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Abstract

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In this loboratory, the pulsed-power vertical gel electrophoresis system produced by ORTEC has provided superior resolution of Neurospora proteins, but recovery of SAICAR synthetase (Fisher 1969 Biochim. Biophys. Acta 178: 380) activity has been quite low. The data of Fantes and Furminger (1967 Nature 215: 750) indicated that the persulfate normally used in the ORTEC procedure might be deleterious to enzyme activity. Due to the low concentration of methylene-bisacrylamide used in the ORTEC procedure, it is not possible to substitute riboflavin directly for persulfate as the catalyst because the resulting gels ore not firm enough to be usable.

Two modifications hove proven satisfactory in the separation of Neurospora proteins. Both give much greater recovery of enzyme activity. The first modification is simply to increase the acrylamide monomer concentration in the gel layers from the usual 8, 6, and 4.5% to 10, 7.5, and 6% with no modification of the bisacrylamide concentration. The second modification involves a doubling of the bisacrylamide concentration from 2.5% to 5% of the acrylamide monomer concentration with no alterations of the monomer concentrations. Changing the bisacrylamide concentration has the additional advantage of making the gels much easier to remove from the electrophoresis cell.

With both modifications, equal volumes of 0.00025% riboflavin (freshly prepored from a 10X stock stored in the dark under refrigeration) were substituted for persulfate, and photopolymerization was allowed to take place for 20 min. with two 15-watt daylight fluorescent tuber as close as possible to the gels.

These methods ore compatible with the previously described fluorescent staining (see preceding communication): the level of riboflavin fluorescence remaining after photopolymerization is low and riboflavin which does remain is carried through the gel close to the leading-ion front, thus removing it from the area of protein bonding. (This research was sponsored by the U. S. Atomic Energy Commission under contract with Union Carbide Corporation and by the AEC Postdoctoral Fellowship Program of the Oak Ridge Associated Universities). • • • Biology Division, Oak Ridge National Laboratory, Oak Ridge, Tennessee 37830.